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THE EFFECTS OF A LACK OF OXYGEN AND OF LOW OXYGEN TENSIONS ON PARAMECIUM

J. A. KITCHING[1]

(*From the Department of Physiology, Princeton University, Princeton, N. J., and the Department of Zoölogy, University of Bristol, England*)

INTRODUCTION

The ability of *Paramecium* to survive without oxygen has been the subject of many investigations, and the results previously published are at variance with one another. Loeb and Hardesty (1895) confined *Paramecium* in a special gas chamber which they freed of oxygen either with a stream of hydrogen or by means of alkaline pyrogallol; the organisms died after twenty-four hours. Budgett (1898), using an Engelmann gas chamber flushed with hydrogen, obtained a survival time of several hours, after which the *Paramecium* blistered. Pütter (1905) found *Paramecium caudatum* to survive for five or six days in a specially designed bottle which had been flushed thoroughly with nitrogen. Fauré-Fremiet et al. (1929) got a survival time of twenty-four hours at 25° C. in sealed tubes containing leuco-methylene blue (reduced by sodium hydrosulphite). Juday (1909) discovered *Paramecium* sp. in the deeper waters of Lake Mendota (Wisconsin) at a time when he believed these waters to be devoid of free oxygen; according to him *Paramecium* is therefore able to live anaerobically for several months. Fortner (1924) enclosed *P. caudatum* in an atmosphere of hydrogen together with aerobic bacteria to remove any remaining oxygen; they survived for several hours, and their contractile vacuoles continued to function, although at a reduced frequency. Gersch (1937) found that *Paramecium* died within 10 seconds in gas purified of its oxygen by means of alkaline pyrogallol. In the work which is described in this paper I have attempted to harmonize these discrepancies.

In addition it has been claimed by several investigators that the respiration of *Paramecium* is insensitive to cyanide (Lund, 1918; Gerard and Hyman, 1931; Shoup and Boykin, 1931). I have therefore made some observations to determine the capacity of *Paramecium* to carry on its normal activities, such as secretion by the contractile vacuoles and locomotion, in the presence of cyanide.

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[1] Fellow of the Rockefeller Foundation.

APPARATUS AND METHODS

Anaerobiosis in a Hanging Drop

In subjecting Protozoa to a lack of oxygen, it is necessary to ensure the following conditions:

(1) A genuine lack of oxygen. It is not possible to assure complete absence of oxygen molecules, but the oxygen content of the medium surrounding the organisms must be so small that the organisms could not possibly make any significant use of it for oxidative metabolism. Oxygen must not be generated by accompanying plant cells or bacteria.

(2) No extraneous contamination. For instance, there must be no harmful impurities in any gas used. For this reason the absorption of oxygen by alkaline pyrogallol is perhaps to be avoided.

(3) No harmful secondary effects. For instance, the flushing of the medium with an oxygen-free gas mixture must not result in a harmful shift of the hydrogen ion concentration.

The general method used was to pass oxygen-free gas past a hanging drop containing the organisms. For this purpose cylinder hydrogen, or cylinder nitrogen (water-pumped), was first bubbled through concentrated sulphuric acid, dilute sulphuric acid, dilute potassium hydroxide and distilled water. Next it was purified of its oxygen. In most experiments hydrogen was used, and was passed through an electrically heated quartz tube containing platinized asbestos. The quartz tube extended sufficiently far at each end beyond the heating coil to avoid any significant warming of the deKhotinsky cement seals. In some experiments, however, nitrogen was passed over hot copper in a special internally heated furnace described by Kendall (1931). This furnace consists of a Pyrex glass tube containing a cylinder of copper gauze of large surface area, which is heated from within by means of a coil of nichrome wire. In this furnace it is always possible to see the condition of the copper, and in the time required for one experiment only a small section of the copper, at the inflow end, became tarnished. Finally the gas was carried by pure lead tubing with seals of deKhotinsky cement through a closed glass wash tube with distilled water in it to the chamber containing the organisms.

The organisms were mounted in a hanging drop on a coverglass which was sealed with vaseline or a mixture of vaseline and paraffin wax to the chamber. The hanging drop was not allowed to touch the vaseline. The chamber itself consisted of a glass ring about 2 cm. in diameter and 1 cm. deep, closed underneath by a microscope slide to which it was sealed with deKhotinsky cement, and with glass inlet and outlet tubes. The upper edge of the ring was ground to support a coverglass, and the microscope slide which formed the base of the chamber fitted onto the mechanical stage of the microscope.

From the chamber the gas was carried by lead tubing to a light-proof box, where it was bubbled either through a simple water trap or through a suspension of marine luminous bacteria. These bacteria (*Achromobacter fischeri*) luminesce in the presence of minute traces of oxygen, and according to Harvey and Morrison (1923) about 0.005 mm. of oxygen can be detected in this way. After the purified hydrogen or nitrogen had bubbled for five or ten minutes through the bacterial suspension no luminescence could be detected with the dark-adapted eye, but admission of air gave immediate recovery of luminescence. An analysis of the purified gas made by mass spectrograph by Dr. W. R. Guyer showed no trace of oxygen, although one part in 10^5 could have been detected.

For control experiments another glass chamber, similar to the first, was mounted on the stage of a second microscope, and CO_2-free air was drawn through gently with a suction pump.

Anaerobiosis in a Tube with Reduced Redox Indicator

The survival of organisms in the presence of a reduced redox indicator low in the redox scale has been used as a demonstration of anaerobic life. For instance, Clark (1924) found that certain bacteria reduced indigo carmine until a high ratio of the reduced to the oxidized substance was attained. He calculated that the oxygen tension in equilibrium with this mixture was so low as to be physically meaningless. For the present purpose, however, such a condition does not establish anaerobiosis, since the bacteria were obtaining oxidative energy by reducing the dye. The dye should therefore be reduced as completely as possible to avoid this possibility. Furthermore, the use of methylene blue, indophenols, or other indicators high in the series is to be avoided. The reoxidation of indicators high in the series is relatively slow at atmospheric oxygen tension (Barron, 1932), and might be very slow at low oxygen tensions even at the experimental hydrogen ion concentration. Harvey (1929) has shown that indophenols in the presence of luminous bacteria may remain reduced in the presence of a concentration of free oxygen sufficient to allow the bacteria to luminesce. Under certain conditions equilibrium may never be attained, and the state of the dye is then no indication of the oxygen tension in the solution. The method described below to the best of my knowledge avoids these errors.

Paramecium in the requisite medium was placed in an internally sealed glass wash tube with some platinized pumice and sufficient indigotrisulphonate to color the solution a clear blue. (Platinized pumice was found more satisfactory than platinized asbestos, as the latter is easily stirred up, and splits into fine sharp spikes which may damage the organisms. Platinized pumice was prepared by boiling pumice chips in chloroplatinic acid until most of the air had been driven out of the pores. Then sodium formate was added until platinum was precipitated in the pores and all over the surface of the pumice. The chips were then washed very thoroughly in running water.) The wash tube was sealed with deKhotinsky cement to the source of purified hydrogen, and the gas escaping from it was carried by lead tubing to the anaerobic chamber for a parallel experiment. The indicator dye bleached five or ten minutes åfter the hydrogen was turned on. The wash tube was then wrapped completely and thoroughly in black cloth so as to exclude all light, and slow bubbling of the hydrogen was continued. This precaution was taken although no photosynthetic organisms were ever detected in the cultures.

Controlled Oxygen Tensions

In order to subject *Paramecium* to controlled and known oxygen tensions, the apparatus used for anaerobiosis in a hanging drop was modified. Hydrogen, purified of its oxygen as already described, and oxygen were led through flow meters (see Harvey and Morrison, 1923) to a T tap in which they were mixed. The resulting known gas mixture was then conveyed through the wash tube with distilled water to the observation chamber. Lead tubing and seals of deKhotinsky cement were used throughout. According to a later refinement, condensation of water in the capillaries of the flow meters was prevented by a tubular show-case lamp placed alongside them. By manipulation of the T tap the organism could be subjected rapidly either to pure hydrogen or to any desired ratio of hydrogen and oxygen.

In a few experiments at very low oxygen tensions, oxygen was mixed with hydrogen in proportions measured by flow meters, and a small part of the resulting mixture was then mixed in the same way with additional pure hydrogen. The unwanted part of the first mixture was rejected through a mercury trap. In this way extremely low tensions of oxygen could be provided with reasonable accuracy.

The tension of oxygen in the observation chamber was calculated with due regard for the barometric and water vapor pressures. The total gas pressure in the observation chamber exceeded that of the air by an insignificant amount.

The Diffusion of Oxygen in a Hanging Drop

I am indebted to Dr. H. P. Robertson of the Physics Department, Princeton University, for a mathematical expression relating the thickness of the hanging drop, the original concentration of oxygen in it, the diffusion coefficient of oxygen in water, and the time required to bring that drop to any given low oxygen tension after the drop has been placed over an atmosphere devoid of oxygen. The drop has been regarded as a film parallel to the coverglass, which for my experiments is reasonably true. Of the infinite series which was derived only the first term is significant, viz.

$$t \sim -\frac{4b^2}{\Delta\pi^2} \cdot \log_e \frac{\theta\pi^2}{8},$$

where t = the time required for the equilibration,
b = the thickness of the drop,
θ = the ratio of concentration of oxygen attained at time t to initial concentration,
Δ = the diffusion coefficient of oxygen in water.

For conditions approaching those of the experiments Δ is given by Bruins (1929) as very nearly 2.0×10^{-5}.

It will be seen that the time required for the drop to reach any given oxygen tension varies as the square of its thickness. Let us choose arbitrarily an oxygen tension of $10^{-4} \times$ the oxygen tension of the atmosphere—less than one-tenth the minimal value necessary (as will be shown later) to allow activity in *Paramecium.* For $\theta = 10^{-4}$, when $b = \frac{1}{4}$ mm., $t = 2$ minutes; and when $b = \frac{1}{2}$ mm., $t = 8$ minutes. The films used were of this order of thickness, and in practice it is probable that equilibration is accelerated by the water currents set up by the swimming of the *Paramecium,* as well as by convection currents.

These theoretical results agree well with experiments on certain marine amoebae, to be published later, in which oxygen tensions below ½ mm. are required for stoppage of movement, and which in a hanging drop come to a standstill within five minutes of the time when pure hydrogen is turned on. It may be concluded that in my experiments adequately anaerobic conditions were attained within ten minutes. Similarly the equilibration of a hanging drop with gas mixtures of known low oxygen tension must be rapid, provided always that the drop is a thin one.

MATERIAL

Paramecium was cultured in timothy hay infusion, and was obtained from the following sources:

(1) *P. multimicronucleatum* collected from a backwater of the canal near Princeton, N. J. Individuals of this race had three contractile vacuoles.
(2) *P. multimicronucleatum* of a pure line (Clone I), with two contractile vacuoles.
(3) *P. caudatum* of a pure line (Clone D).

TABLE I

Survival of *Paramecium multimicronucleatum* clone I under anoxic conditions in a hanging drop of its own culture fluid. CO_2, when used, was at a partial pressure of 12 mm., which was sufficient to maintain the culture fluid at pH 7.1 approximately. Room temperature was 24–28°C.

Gas used	Time for first *Paramecium* to blister	Time for fifty per cent to blister	Time for last to blister	Number of animals used	Date (1939)
	minutes	*minutes*	*minutes*		
Pure H_2	160	370	440	14	August 6
Pure N_2	210	465	585	43	August 3
$H_2 + CO_2$	360	650	690	19	July 29
$H_2 + CO_2$	595	665	710	28	August 1
$N_2 + CO_2$	500	660	720	12	August 5
$N_2 + CO_2$*	500	660	725	9	August 7
CO_2-free air (control experiment)....	—†	—	—	21	July 30–31

* Extra pure CO_2: see text.
† All survived throughout experiment (31 hours) and remained normal in appearance and activity.

The last two were both kindly supplied to me through the courtesy of Professor S. O. Mast of the Johns Hopkins University.

RESULTS

In all cases and in all media *Paramecium multimicronucleatum* and *P. caudatum*, when mounted in a hanging drop in absence of oxygen, continued to swim and to evacuate fluid by the contractile vacuoles for a limited time. However, the speed of swimming and the rate of output of the contractile vacuoles decreased, and eventually the organisms stopped. Then the trichocysts were discharged; the anterior end of the body usually, but not always, became constricted; blisters formed at the surface of the body; and cytolysis ensued. *Paramecium* which had ceased all activity for lack of oxygen, and which had even begun to blister, recovered rapidly on admission of sufficient oxygen (see Tables III and IV).

The duration of anaerobic survival was very variable, and depended partly on the media used. The longest survival times were got with culture fluid. In hanging drops of culture fluid survival times in absence of oxygen ranged from one to twelve hours. It appeared (as already shown by Pütter (1905)) that *Paramecium* from well-fed, flourishing cultures survived the longest. There were considerable differences between cultures of the same clone. However, exposure of a hanging drop of culture medium to pure hydrogen or nitrogen leads to a loss of carbon dioxide, with a resulting shift of the hydrogen ion concentration far into the alkaline range. Accordingly, pure hydrogen was bubbled through some culture fluid and the resulting shift of pH, according to measurements kindly made for me with the glass electrode by Dr. Marshall E. Smith, was from about 6.2–6.4 to about 8.9–9.0. Addition of about 12 mm. partial pressure of carbon dioxide to the hydrogen was sufficient to maintain the culture fluid at about pH 7.1. When hydrogen or nitrogen together with this amount of carbon dioxide were passed through the observation chamber, the *Paramecium* survived slightly but significantly longer than without the carbon dioxide (Table I); the series of changes leading to death was, however,

TABLE II

The effect of a lack of oxygen on *Paramecium caudatum* clone D, after segregation in test tube in culture fluid without hay.

Medium	Culture fluid; pure hydrogen	M/300 phosphate buffer; pure H_2	M/300 phosphate buffer; CO_2-free air as control
Experiments on same day:			
Time required for first one to stop or blister, in minutes	88	23	no visible adverse effects
Same for 50 per cent	154	56	—
Same for last one	235	76	—
Number of individuals used	28	8	12
Experiments five days later:			
Time required for first one to stop or blister	79	7	27
Same for 50 per cent	79	7	29
Same for last one	109	9	68
Number of individuals used	8	4	7

the same. In these experiments no purification of the carbon dioxide was undertaken, but the connection bewteen the carbon dioxide cylinder and the apparatus was entirely of glass, lead tubing, and deKhotinsky cement, and the pressure was regulated by a double water trap of such a nature as to prevent backward diffusion of oxygen. The manufacturers of the carbon dioxide stated that the oxygen content of their cylinders varied between two and sixteen parts in ten thousand. The oxygen content of the mixed carbon dioxide and nitrogen or hydrogen must have been insignificantly low, and experiments with a cylinder which had been cleaned out and filled with special care by the manufacturers gave entirely similar results.

Table III

The effect of distilled water, as compared with culture fluid, on the ability of *Paramecium multimicronucleatum* to withstand a lack of oxygen. For each gas mixture two separate hanging drops, with the two media, were suspended from the same coverglass.

Time	Pure Hydrogen		CO_2-free Air (Control)	
	Culture fluid	Distilled water	Culture fluid	Distilled water
minutes				
0	started	started	started	started
57	all very slow	1 blistered, rest very slow	normal movement	normal movement
64	1 blistered, rest very slow	"	"	"
73	all stopped	all stopped	"	"
74	3.5 mm. oxygen admitted			
80	3 swimming, rest appear dead	3 swimming. rest appear dead	"	"
395	2 swimming normally, rest dead	2 swimming normally, rest dead	"	"
Number of organisms used	15	12	12	8

The results obtained in the presence of a reduced redox indicator (indigo trisulphonate) in a wash tube in the dark agreed well with those obtained by the hanging drop method. In several experiments the purified hydrogen was bubbled first through the wash tube with the platinized pumice, and then through the anaerobic chamber. In all such experiments a few individuals survived in the wash tube after all had cytolysed in the anaerobic chamber. However, the wash tube contained many thousands of individuals, and it is to be expected that out of so many a few would be more hardy. At the time when the majority in the anaerobic chamber cytolysed there was a marked decrease in the number visible in the wash tube. The longest survival in culture fluid was about twelve hours.

In other experiments the organisms were washed four times with M/300 phosphate buffer ($Na_2HPO_4 + KH_2PO_4$) at pH 7.0–7.1, and mounted over the anaerobic chamber in this medium. Under anoxic conditions *Paramecium* underwent the same series of changes as in culture medium, although the constriction of the anterior end and the blistering seemed more sudden and violent. Also it survived for much less time in phosphate buffer than in culture fluid; even though in air it lived without apparent damage for many hours (in some cases observations were extended over two days) in the buffer solution. However, if some *Paramecium* were removed from the culture and placed in a test tube with some of the culture fluid but without hay, their ability to survive anaerobically in either medium (culture or buffer solution) decreased progressively, and after a few days they became fatally susceptible to phosphate buffer even when in air. These results are illustrated in Table II.

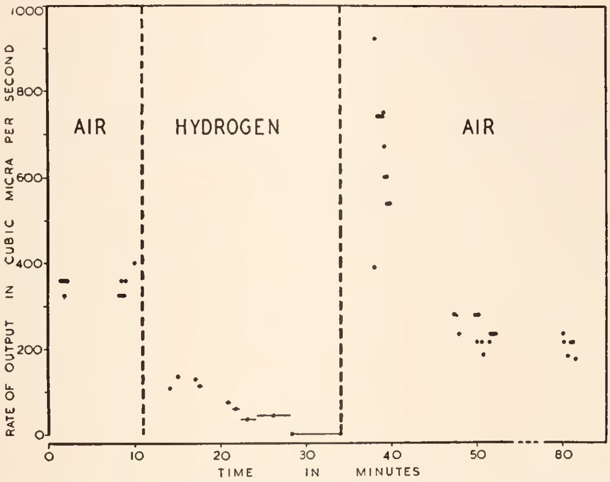

Fig. 1. The effect of pure hydrogen on the rate of output of the anterior contractile vacuole of *Paramecium multimicronucleatum* in a hanging drop of dilute phosphate buffer.

In the absence of oxygen *Paramecium* was found (in a few experiments) to survive equally well either in culture fluid or in distilled water (Table III). However, in M/300 KCl some individuals cytolysed almost instantly in air, and in absence of oxygen the remainder survived only a few minutes.

The contractile vacuoles, both in culture medium and in phosphate buffer, continued to function under anaerobic conditions, but finally became very slow. In two experiments *Paramecium* was slowed down by means of an agar gel just sufficiently viscous to make observation possible. The result of one of these experiments is plotted in Fig. 1. The stoppage of the contractile vacuoles after a period of lack of oxygen was found to be reversible. Contrary to the findings of Frisch (1937),

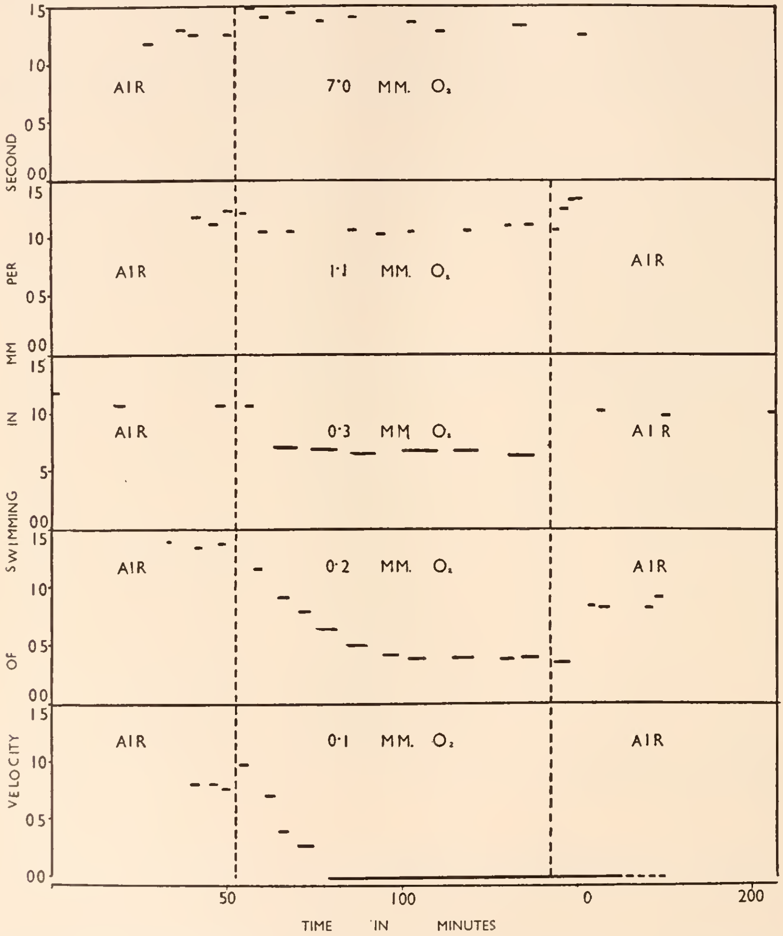

FIG. 2. The influence of low oxygen tensions on the rate of swimming of *Paramecium multimicronucleatum* in dilute phosphate buffer.

the *Paramecium* which I used showed normal activity of the contractile vacuoles whether they were swimming or stationary. This was true of *Paramecium* in hanging drops of culture medium or dilute buffer in contact with air, with or (usually) without agar. I attribute the discrepancy to the fact that his organisms had been sealed in a vaseline ring for many days and were not fully active.

A series of experiments was made to determine the survival and activity of *Paramecium multimicronucleatum* at low oxygen tensions. The rate of swimming of healthy *Paramecium* in phosphate buffer was found, after an initial burst of high activity, to remain reasonably constant for as long as observations were continued (up to 22 hours). Accordingly single organisms were acclimatized for 90 minutes in a hanging drop of the buffer solution in a stream of moist carbon dioxide-free air. After this observations were made of the rate of swimming (*a*) in carbon dioxide-free air, (*b*) in a known mixture of oxygen and hydrogen, and (*c*) in carbon dioxide-free air. The second period (*b*) lasted usually about two hours. Readings of the time required for the organisms to traverse the distance indicated by the ocular scale (2.06 mm.) were made in groups of twelve, and the mean for each group determined. The standard error of the mean time usually lay between 3 and 5 per cent of the mean value. Results are shown in Fig. 2. There was a slowing down of swimming in oxygen tensions below 1 mm. Below 0.2 mm. approximately the organisms died, but above this value

TABLE IV

Recovery of *Paramecium caudatum* at known oxygen tensions from lack of oxygen, in dilute phosphate buffer solution.

Duration of lack of oxygen in minutes	Condition at end of anaerobic period: number of animals		Oxygen admitted in mm.	Duration of observations at this tension in minutes	Condition after admission of oxygen at this tension: number of animals		
	Stopped but normal in shape	Stopped but pointed and blistered			Recovered after stoppage	Recovered after stoppage and blisters	Cytolysed
44	1	2	27	36	1	1	1
46	6	2	7.0	38	6	1	1
31	5	2	0.28	21	3	1	3
36	1	7	1.4	38	1	6	1
31	1	5	0.85	41	1	1	4
57	3	2	0.57	22	3	0	2
53	11	4	0.23	24	1*	0	14
36	3	1	0.17	18	0	0	4

* Movement very slow.

they slowed down to a speed approximately constant within the duration of the treatment; and this effect was reversible. Individual variation was such that it did not seem worthwhile to try to determine a detailed relation between oxygen tension and rate of swimming.

In a further series of experiments a group of *Paramecium* was mounted in a hanging drop of the dilute phosphate buffer in the anaerobic chamber, and subjected to anoxic conditions until all had stopped and some of them had blistered. Then oxygen was admitted at a known partial pressure, and the extent of recovery recorded. Results are shown briefly in Table IV. Recovery could be obtained, on admission of sufficient oxygen, even after the organisms had begun to blister, and in such cases the blisters were gradually resorbed. In many of these cases it is certain that if the organisms had been left for another one or two minutes without oxygen irreversible cytolysis would have ensued. The minimal oxygen tension needed for recovery was of the same order as that which was found just to allow swimming in the previous series of experiments.

The effect of cyanide on the secretory activity of the contractile vacuoles and on the general activity of the animals was examined briefly in three experiments. Single individuals of *Paramecium multimicronucleatum* were mounted in a hanging film of river water just sufficiently thin to prevent too rapid swimming. The cover glass was sealed with vaseline over a small glass cell half filled with river water, so that evaporation from the film was prevented. After a period of examination in river water the *Paramecium* was transferred with the usual four washes to a dilute solution of sodium cyanide (M/200, M/1,000, M/2,000, pH corrected to 7.1) in river water, and the fluid in the cell was also replaced by the cyanide solution. This procedure was carried out as quickly as possible in order to avoid loss of cyanide, and the examination of the organism was continued. After an initial depression of activity the vacuoles continued to function regularly although slightly less vigorously than in plain river water. The cilia also continued to beat actively. Observations were continued in one case for over ten hours after the cyanide treatment was begun.

DISCUSSION

The majority of workers are agreed that *Paramecium* at room temperatures (20–25° C.) can survive for some hours, though not days, without oxygen. In view of the indubitable demonstrations of this fact, the results of Gersch (1937), who observed almost instantaneous death, must be discounted. It seems somewhat questionable whether Pütter, who (1905) obtained a much longer supposedly anaerobic survival, really achieved strictly anoxic conditions; and it is clear that *Paramecium* can make some use of oxygen at partial pressures below 1 mm. It also seems doubtful whether Juday (1909) would have detected oxygen in such low concentrations in the bottom waters of Lake Mendota (Wisconsin), where he claimed that *Paramecium* lived anaerobically. However, it remains possible that *Paramecium* might be found to survive without oxygen for longer periods under experimental conditions if it were supplied with suitable food. Slight discrepancies between the results of other workers may be ascribed to variations in the excellence of the oxygen "lack," possibly to the use of several different species, and particularly to variations in the state of nutrition of the organisms. This latter condition was stressed by Pütter (1905), and probably accounts for the beneficial effect of stirring the culture.

There is clearly some adverse influence in phosphate buffer which a normal healthy *Paramecium* can withstand in air, but which successfully operates against a starved *Paramecium* in air or against a well-fed one in absence of oxygen. The constriction of the anterior end of the organism and the blistering just before death are probably the result of a violent contraction of the myonemes. They were found to occur even when the organism was in M/20 lactose solution, and it is therefore unlikely that they can be attributed to osmotic uptake of water by the organism. A somewhat similar effect has been seen in *Paramecium* subjected to an electric current (see Kalmus, 1931). The phosphate buffer, either directly or indirectly, hastens the time for the myoneme contraction. It seems probable that the harmful effect of this buffer is due to a lack of balance of ionic concentrations, although this matter has not yet been investigated in detail.

Whereas cyanide in very low concentration inhibits the action of the contractile vacuoles of peritrich ciliates (Kitching, 1936), it has no very marked effect on those of *Paramecium*. The prolonged anaerobic activity of *Paramecium* might account for this. However, according to the results of various workers, at least a considerable part of the respiration of *Paramecium* must be insensitive to cyanide. Gerard and Hyman (1931) found that substitution of phosphate buffer for a calcium-containing water approximately halved the rate of oxygen consumption, but addition of cyanide made no further difference. The persistence of vacuolar activity in the presence of cyanide harmonises with the view that the respiration of *Paramecium* is relatively insensitive to this substance.

Paramecium is able to continue swimming, or to recover from a lack of oxygen, at oxygen tensions down to 0.3 mm. However, it is possible that higher tensions may be needed for growth and prolonged survival.

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Amberson (1928), by a not very delicate method, obtained results which suggest that the respiration of *P. caudatum* is depressed slightly at tensions of oxygen below 50 mm.

A comparison of the results obtained with *Paramecium* and with other Protozoa will be made in another paper.

ACKNOWLEDGMENTS

I wish to thank Dr. E. N. Harvey for his guidance and criticism, as well as for the facilities of the physiological laboratory, Princeton University. The work was completed at the Marine Biological Laboratory, Woods Hole.

SUMMARY

1. *Paramecium multimicronucleatum* and *P. caudatum* were subjected to pure hydrogen, pure nitrogen, and known mixtures of hydrogen and oxygen, while in a thin hanging drop under microscopical observation.

2. In all cases there was a limited period of anaerobic survival, during which activity of swimming and of contractile vacuoles was gradually diminished. Finally the organisms stopped, blistered, and cytolysed.

3. Admission of sufficient oxygen, even after blistering had begun, gave recovery.

4. Survival under anoxic conditions was best in culture fluid maintained at a reasonable hydrogen ion concentration by the addition of small quantities of carbon dioxide to the hydrogen or nitrogen. Under these conditions some organisms survived as long as twelve hours.

5. *Paramecium* was extremely variable as regards the length of anaerobic survival in any one medium. This variability is ascribed to physiological condition and not to genetic factors.

6. Survival under anoxic conditions was much shorter in dilute phosphate buffer than in culture medium. This is tentatively ascribed to a lack of balance of ionic concentrations.

7. In phosphate buffer the rate of swimming was reduced at tensions of oxygen below 1 mm., and the organisms died within a short time at tensions below 0.2 mm. At tensions above 0.3 mm. some measure of recovery from lack of oxygen could be obtained.

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